DMC Admin file

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

P940035

Date JUL - 2 1996

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Matritech, Inc.
Matritech NMP22™ Test Kit - ACTION

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

Prepared by Peter E. Maxim, Ph.D., CDRH, HFZ-440, 6/14/96, 594-1293

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DRAFT

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. _____]

Matritech Inc.; Premarket Approval Of Matritech NMP22™ Test Kit

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Matritech Inc., Newton, MA, for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of Matritech NMP22™ Test Kit. After reviewing the recommendation of the Immunology Devices Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on July 2, 1996, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., Rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Peter E. Maxim, Ph.D.,

Center for Devices and Radiological Health (HFZ-440),

Food and Drug Administration,

9200 Corporate Blvd.,

Rockville, MD 20850,

301-594-1293.

SUPPLEMENTARY INFORMATION: On November 7, 1994, Matritech, Inc., Newton, MA 02160, submitted to CDRH an application for premarket approval of Matritech NMP22™ Test Kit. The Matritech NMP22™ Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of nuclear matrix protein NMP22 in stabilized voided urine. The Matritech NMP22™Test Kit is indicated as an aid in the management of patients with transitional cell carcinoma of the urinary tract (TCC/UT), after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT. The Matritech NMP22™ Urine Collection Kit, intended for the collection, stabilization, and transport of human urine which will be tested using the Matritech NMP22™ Test Kit.

On November 30, 1995, the Immunology Devices Panel, an FDA advisory committee, reviewed and recommended approval of the application.

On July 2, 1996, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity For Administrative Review

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), (21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Cheryl L. Hayden, Ph.D.
Director, Clinical and Regulatory Affairs
Matritech Inc.
330 Nevada Street
Newton, Massachusetts 02160

JUL - 2 1996

Re: P940035
. Matritech NMP22™ Test Kit
Filed: November 7, 1994
Amended: June 15, August 2, October 26, December 1, 1995;
February 2, February 14, and June 18, 1996

Dear Dr. Hayden:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Matritech NMP22™ Test Kit. The Matritech NMP22™ Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of nuclear matrix protein NMP22 in stabilized voided urine. The Matritech NMP22™ Test Kit is indicated as an aid in the management of patients with transitional cell carcinoma of the urinary tract (TCC/UT), after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

Expiration dating for this device has been established and approved at 15 months at 2° to 8° C. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g)

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of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Peter E. Maxim, Ph.D. at (301) 594-1293.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Cheryl L. Hayden, Ph.D.
Director, Clinical and Regulatory Affairs
Matritech Inc.
330 Nevada Street
Newton, Massachusetts 02160

AUG - 6 1996

Re: P940035
Matritech NMP22™ Test Kit
Filed: November 7, 1994
Amended: June 15, August 2, October 26, December 1, 1995,
February 2, February 14, and June 18, 1996

Dear Dr. Hayden:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) completed its evaluation of your premarket approval application (PMA). We notified you that the premarket approval application (PMA) for the Matritech NMP22™ Test Kit was approved by an approval order dated, July 2, 1996. The following approval, subject to conditions, as described in that approval order:

> The Matritech NMP22™ Urine Collection Kit, intended for the collection, stabilization, and transport of human urine which will be tested using the Matritech NMP22™ Test Kit.

was omitted. We hope that this omission has not inconveniencied you. If you have any questions about this corrective action, please contact me at (301) 594-3084.

Sincerely yours,

Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical
Laboratory Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

SUMMARY OF SAFETY AND EFFECTIVENESS

I. General Information

Device Generic Name: Tumor Associated Antigen Immunoassay System

Device Trade Name: Matritech NMP22™ Test Kit

Applicant's Name and Address: Matritech, Inc.
330 Nevada Street
Newton, MA 02160

Premarket Approval Application (PMA) Number: P940035

Date of Panel Recommendations: November 30, 1995

Date of Notice of Approval of Application: JUL - 2 1996

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II. Indications for Use

Intended Use

The Matritech NMP22™ Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of nuclear matrix protein NMP22 in stabilized voided urine. The Matritech NMP22™ Test Kit is indicated as an aid in the management of patients with transitional cell carcinoma of the urinary tract (TCC/UT), after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT.

The Matritech NMP22™ Urine Collection Kit, intended for the collection, stabilization, and transport of human urine which will be tested using the Matritech NMP22™ Test Kit.

Contraindications, Warnings and Precautions:

There are no know contraindications for the NMP22™ Test Kit. See attached labeling for Warnings and Precautions.

Since urinary levels of NMP22 may be elevated in some non-malignant conditions such as urinary tract infections or interstitial cystitis, samples should not be collected until the condition has been effectively treated. NMP22 levels may also be elevated as a result of instrumentation of the urinary tract, therefore, samples should not be collected within 5 days of a procedure such as cystoscopy or catheterization. Elevated levels of urinary NMP22™ have been documented in patients undergoing systemic chemotherapy and in patients who have undergone complete cystectomy; significance of these results is unknown. Elevated levels of urinary NMP22 are seen occasionally following extreme exercise such as running 10 miles or more. Low urinary levels of NMP22 are not necessarily indicative of absence of malignant disease in the urinary tract.

III. Device Description

Nuclear matrix proteins (NMP) make up the internal structural framework of the nucleus[1,2] and are associated with such functions as DNA replication, RNA synthesis, and hormone binding.[3,4,5] Further work has indicated that NMPs are involved in regulation and coordination of gene expression.[4,6,7] Later work by Fey and Penman[8] demonstrated that NMP expression varied with cell type of origin. This observation was followed by work showing that soluble NMPs could be detected in the serum of cancer patients in higher concentrations than were found in normal serum.[9] Partin and colleagues[10] demonstrated that the pattern of expression of NMP differed in normal prostate tissue, benign prostatic hyperplasia, and prostate cancer. Previous work has identified specific NMPs to osteosarcoma[11], colon[12] and breast cancer[13]. These observations indicated that measurement of NMP may have clinical utility in the management of a number of malignancies.

The antibodies contained within this assay recognize the head and rod domains of NuMA, nuclear mitotic apparatus protein. NuMA has been shown to be present in malignant tissues at levels more than ten times higher than in normal tissues. The NuMA antigen moiety detected

by the Matritech NMP22™ Test Kit is referred to as NMP22. In the urine of healthy individuals, NMP22™ is present at low levels. The majority of patients with bladder cancer have been shown to release large quantities of NMP22™ into the urine. The assay is designed to quantify NMP22™ in stabilized voided urine. Patients with TCC/UT present in the urinary tract have been shown to release higher levels of NMP22™ into the urine. This assay is designed to quantify NMP22™ in stabilized voided urine.

The Matritech NMP22™ Test Kit is an enzyme immunoassay in a 96 well microtiter strip-well format. The assay employs two murine monoclonal antibodies that are specific for the nuclear matrix protein NMP22.

Calibrators, controls, and stabilized patient urine react with an antibody coated onto wells of a microtiter plate. After washing, the captured NMP22 antigen reacts with a second antibody labeled with digoxigenin. After a wash, the digoxigenin-labeled antibody is detected with an anti-digoxigenin antibody labeled with horseradish peroxidase using o-phenylenediamine substrate.

The reaction is terminated by the addition of 2 molar sulfuric acid. The concentration of antigen in the urine is proportional to the intensity of color development and the actual concentration of NMP22 is determined from a standard curve. The standard curve is constructed from the concurrent testing of the NMP22™ Urine Calibrators which range in concentration from 0 to approximately 120 U/mL.

IV. Alternative Practices and Procedures

Current methods for diagnosis and monitoring of tumors of the bladder and urethra include cystoscopic examination and cytopathologic examination of cells in voided urine or bladder washings. Current methods for diagnosis and monitoring of tumors in the ureters or renal pelvis include endoscopic examination and intravenous pyelography. Definitive diagnosis for all tumors of the urinary tract requires pathologic examination of biopsy material. In addition, routine medical practices and procedures might include: physical examination, radiographic examination, ultrasound scan, computer assisted tomography (CT) scan, lymphangiography, and other procedures for overall clinical evaluation of the patient.

V. Marketing History

The Matritech NMP22 Test Kit has been marketed without withdrawal, in Japan, Germany, and Canada.

VI. Potential Adverse Effects of The Device on Health

When the Matritech NMP22™ Test Kit is used as indicated, the adverse effects on the health of the patients being evaluated for transitional cell carcinoma of the urinary tract are associated with a false positive or negative test result. A false positive result may lead to more aggressive follow up procedures and possibly the initiation of therapy. A false negative result might lead to a delay in patient treatment.

VII. Summary of Studies

A. Nonclinical Laboratory Studies.

Antigen.

NMP22 is a fragment of NuMA, a 236 kD polypeptide that constitutes less than 5 percent of the nuclear matrix proteins. NuMA has been identified as a component of the nuclear matrix that redistributes to the mitotic spindle on mitosis. NuMA has been sequenced and expressed and consists of a long alpha helical region flanked by two globular domains. Molecular sieve chromatography by FPLC showed similar elution profiles of the NMP22™ antigen for both the Calibrator antigen (derived from cultured cell line) and for urine from patients with bladder cancer consisting of two major peaks: > 1 million Daltons and approximately 30,000 Daltons.[1-5]

Antibody

The two mouse monoclonal antibodies used in the NMP22 Test Kit were prepared from an immunization of a nuclear matrix preparation from cultured human tumor cells. The antibodies recognize epitopes that are sensitive to trypsin and per iodate.

Sample Collection

Pre-clinical studies demonstrated that significant void-to-void variations could be seen in the concentration of NMP22 in the urine. In order to reduce this variation and provide an estimate of the average 24 hour urine concentration, a study with a feasibility-stage assay was performed by Matritech to evaluate the optimum patient sampling method for the urine.

It was demonstrated that the average concentration of NMP22 in three randomly voided samples gave a good correlation with the total concentration for 24 hours of urine collection. No correlation was seen with total urinary protein, creatinine, or hemoglobin.

The urine sampling method was determined to be three urine voids within a 24 hour period. The NMP22 value used to evaluate the patient is the average NMP22 value of the three voids.

Reproducibility Studies

The within run and total precision of the NMP22™ Test Kit were determined following procedures outlined in the National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-T2.[14] Three urine samples and three controls were assayed in duplicate in two assays a day for 20 days. The within run precision coefficient of variation (CV) ranged from 1.7 percent to 5.5 percent for the urine samples and from 2.3 percent to 7.0 percent for the controls. The total precision

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(CVs) ranged from 7.7 percent to 9.7 percent for the urine samples and from 4.3 percent to 9.7 percent for the controls. These results are within acceptable limits for a device of this kind.

Recovery

Known concentrations of NMP22 were added to stabilized urine containing low endogenous levels of NMP22. The samples were measured in duplicate. The recovery of NMP22 from a patient's stabilized urine specimen is summarized in Table 1. The mean of 2 assays is reported:

TABLE 1

Concentration added Units NMP22/mL	NMP22 Recovered U/mL	Percent Recovery
48.6	45.9	94
31.5	28.4	90
23.5	22.3	95
11.0	11.4	103
	Overall Recovery	96

The range of individual recoveries of NMP22™ from the stabilized urine of 5 different patients ranged from 89 percent to 111 percent with a mean of 99 percent are within acceptable limits for a device of this type.

Linearity of Dilution

Six stabilized urine samples containing elevated NMP22™ levels were serially diluted with the NMP22 Urine Calibrator #1 (0 U/mL) and assayed in triplicate. Linear regression analysis of NMP22 concentrations measured versus dilution were performed. The slopes for the 6 samples ranged between 0.80 to 1.06 with correlation coefficients of greater than 0.993. These data demonstrated that samples diluted to read within the range of the standard curve would yield accurate measurements of the NMP22 concentrations.

Stability

Zero slope analysis of the data on the kit lot being tested for the longest period of time indicated no statistically significant increasing or decreasing trend for any of the controls or panel members for kits stored for 427 days at 2° C. to 8° C. Stability has been established at 15 months.

Specificity and Interfering Substances

Various substances were evaluated and found not to interfere with the Matritech NMP22™ Test Kit at concentrations listed in Table 2 (concentrations are in mg/dL unless otherwise noted):

TABLE 2

Substance Tested	No Interference with Assay at:
I. Urine Analytes:	
Albumin	100.0 mg/dL
IgG	100.0 mg/dL
Hemoglobin	1.6 mg/dL
Red Blood Cells	1.8 $x10^{11}$ cells/dL
Whole Blood	1.0 %(v/v)
Glucose	20.0 mg/dL
Cytokeratins (TPA U/dL)	40 U/dL
II. Drugs:	
Digoxin	0.05 mg/dL
Acetaminophen	20 mg/dL
Sodium Ascorbate	20 mg/dL
Caffeine	20 mg/dL
Sodium Salicylate	20 mg/dL
Sodium Acetylsalicylate	20 mg/dL
Ampicillin	20 mg/dL
Tetracycline	20 mg/dL
III. Therapeutic Agents:	
BCG (Tice)	5.0 $x10^{6}$ CFU/dL
Thiotepa	60 mg/dL

Limit of Detection

The lowest concentration of NMP22™ antigen that could be reliably measured with the Matritech NMP22™ Test Kit is 2.1 Units/mL. The limit of detection is defined as that NMP22 concentration that corresponds to the absorbance that is two standard deviations above the mean absorbance of twenty replicate determinations of the zero calibrator (0 U/mL).

B. Clinical Investigations

A prospective clinical trial was performed at 14 institutions and at Matritech, Inc., to determine the utility of NMP22™ in the management of patients with TCC/UT and bladder cancers of other histologies. The specific objectives of the study were:

VIII. Specimen Collection and Preparation

A. Specimen Collection

Three separate voids of urine should be collected within a 24 hour period and stabilized immediately, by the patient or medical personnel, using the Matritech NMP22™ Urine Collection Kit, Catalog Number D2000. DO NOT USE OTHER METHODS FOR COLLECTING URINE SAMPLES. INVALID SPECIMEN INFORMATION WILL RESULT FROM IMPROPERLY COLLECTED SAMPLES.

1. Stabilized urine collected with the Matritech NMP22™ Urine Collection Kit should be blue/green in color.
2. Stabilized urine samples processed following Sample Preparation steps B.1.A through B.1.D below may be stored at 2-8°C for up to 1 week prior to measurement. For longer storage time the sample should be frozen at -80°C.
3. Sodium azide or other preservatives should not be added to the samples as invalid specimen information may result when the samples are analyzed in the Matritech NMP22(R) Test Kit.

B. Sample Preparation

1. Prepare a single pooled sample from the three individual voids as follows:
   a) Three separate urine voids each totaling 10mL of blue/green-colored stabilized urine specimen should be received from each patient.
   b) Ensure that each void is at room temperature prior to processing.
   c) Centrifuge the total contents of each individual void in plastic at 500 to 1000 X G for 10 to 15 minutes at 10 to 15°C to remove precipitates.
   d) Decant the supernatant from each void into separate plastic containers.
   e) Pipette an equal volume of supernatant (suggested volume = 0.5mL) from each of the three centrifuged urine voids into a plastic container to create a single pooled sample. Use immediately.
2. Analyze each pooled sample using the Matritech NMP22(R) Test Kit procedure.
3. If desired, individual void samples which have been centrifuged and decanted maybe stored at 2-8°C for up to 1 week prior to measurement. For longer storage times samples should be stored at -80°C.
4. Samples that have been stored, either at 2-8°C or -80°C, should be centrifuged and decanted a second time before use to remove any additional precipitates. DO NOT thaw samples

by placing at elevated temperatures (greater than 25°C). Loss of antigen activity may result.

IX. Procedual Notes

- It is important that the user become familiar with the procedure. It is strongly recommended that the directions be read carefully and understood prior to use.
- A standard curve must be established with every run.
- Disposable pipette tips should be used to prevent cross-specimen or -reagent contamination. It is also recommended to wet pipette tips prior to use.
- To avoid interferences by detergent or other contaminants, all labware must be clean and thoroughly rinsed prior to use.
- To achieve optimal results, adherence to protocol incubation times and temperatures is necessary.
- Avoid microbial contamination of reagents when removing aliquots from the vials. Microbial contamination should be suspected if the reagent solutions become cloudy or emit a stong odor.
- Do not expose OPD reagents to strong light during storage or incubation. Prevent contact of the OPD reagents with any oxidizing reagents or metal.
- Do not place urine samples, or aliquots of calibrators, or controls in any glass containers other than the container in which reagents were supplied.
- During the assay procedure DO NOT allow the microplate strip well to become dry.

X. Quality Control

Good laboratory practices include the use of control specimens within each assay to ensure that all reagents and procedures are performing properly. The Matritech NMP22(R) Test kit contains a set of three controls, which can be used to verify assay performance. Suggested recovery ranges for each control level are printed on the vial labels. Because each laboratory may obtain slightly different results, it is suggested that each laboratory establish its own range for each level of urine control.

XI. Procedure

A. Preparation for Assay

1. Allow kit components and centrifuged stabilized urine samples to equilibrate to room temperature (18-25°C) for 15 to 20 min. before use. Gently swirl all reagents to ensure mixing prior to use.
2. Verify the "pooled" samples have been prepared as outlined in Section VIII. B Sample Preparation.
3. Prepare Wash Solution from Wash Solution 100X Concentrate. Allow Wash Solution 100X Concentrate to equilibrate to room temperature(18-25°C). If any precipitate is present, warm the concentrate until precipitate dissolves. Dilute Wash Solution Concentrate 1:100 with deionized water (10 mL concentrate makes 1000 mL wash solution).
4. Reconstitute each lyophilized Calibrator and Control with 2 mL deionized water. Reconstitute the Sample Diluent with 10 mL of deionized water. Recap and let stand at 18-25°C for approximately 10 minutes. Invert and swirl gently, but do not vortex (avoid foaming). Let stand at least 10 additional minutes before use.
5. Prepare 2M Sulfuric Acid Stop Solution by adding 11.1 mL of concentrated sulfuric acid to approximately 70 mL of deionized water. Bring the stop solution to a final volume of 100 mL with deionized water. Store at room temperature.
6. Open Coated Microplate Strip Well foil package by cutting close to the package edge with the zip-lock strip. Remove strip wells not required for assay from the plate frame and replace with blank strip wells. Make sure strip wells are properly seated in plate frame. Return unused coated strip wells to the foil package along with the desiccant packet. Carefully reseal foil package with the zip lock closure and store at 2-8°C.

B. Assay

1. Wash Strip Well Plate 3 times with Wash Solution (Step XI.A.3) either using an automatic plate washer or manually. For the automatic washing system ensure that the settings allow each well to fill completely (minimum 310uL). Between each wash cycle step, allow for approximately a 10 second soak. After the three cycles are complete ensure the wells are free of residual wash solution by tapping the plate on absorbant paper. Proceed immediately to step 2.
2. Add 200 uL/well of each calibrator, control and pooled sample into assigned duplicate wells. Incubate 2 hours ± 5 min. at 18-25°C.
3. Wash plate 3 times with Wash Solution as described in step 1.

4. Add 200 uL/well of DIG~Anti-NMP22. Incubate 1 hour ± 5 min. at 18-25°C.
5. Wash plate 3 times with Wash Solution as described in step 1.
6. Add 200 ul/well of HRP-SAD. Incubate 30 ± 5 min. at 18-25°C.
7. Transfer required amount of Color Development Buffer(10 mL for half plate, 20 mL for full plate) into a plastic test tube. Add one OPD tablet/10mL of buffer. Cover tube with foil and store in darkened area until ready to use. PREPARE THIS SOLUTION NO MORE THAN 30 MINUTES BEFORE USE. Ensure that the tablet(s) is completely dissolved before use. Avoid contact of metal objects with this solution.
8. Wash plate 3 times with Wash Solution as described in step 1.
9. Swirl OPD solution before use. Add 200 uL/well OPD. Incubate 30 ± 2 min at 18-25°C. PLACE PLATE IN A DARKENED AREA DURING COLOR DEVELOPMENT.
10. Stop reaction with 50 uL/well 2M H_2SO_4 Stop Solution (step XI. A.6). Tap plate gently to ensure total mix of acid with OPD. Wait 10 minutes with plate at 18-25°C. (Storage at this time is also in darkened area.)
11. Read absorbance of each well within 30 minutes of the addition of acid using a spectophotometer that has been set at 490 nm and blanked to zero absorbance with deionized water.

XII. Results

The NMP22 results may be calculated using computer assisted methods or manually on rectilinear graph paper.

A. Computer Assisted Method.

Computer assisted data reduction may be used to calculate results. The performance data in this insert were calculated using a point-to-point curve fit. Curve fitting based on a log-logit relationship is not recommended.

It is recommended that each laboratory select an appropriate curve fit method. The same curve fit method should be used to calculate results from different assay runs to ensure that consistant values will be obtained.

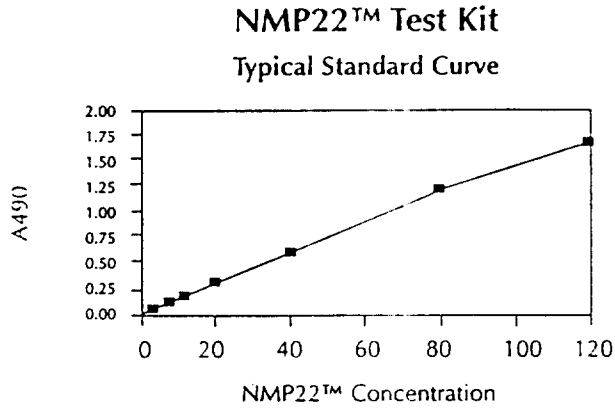
B. Manual Method:

The curve may be constructed manually on rectilinear graph paper by plotting the mean A490 absorbance value for each set of calibrator replicates on the y-axis versus concentration in U/mL on the x-axis. The best curve fit should be drawn through the calibration points. To determine the NMP22 concentration in a patient sample, find the point on the curve corresponding to the mean A490 of the patient sample and drop a vertical line to the

x-axis. Read the concentration of NMP22 in units per milliliter (U/mL).

If the sample has a mean absorbance at 490 nm that is greater than the mean absorbance at 490 nm of the highest calibrator, that sample should be re-run after dilution with the sample diluent to fall within the range of the assay. For diluted samples the NMP22 value obtained in the assay should be multiplied by the dilution factor to obtain the NMP22 concentration in the sample. For example, if a sample was diluted 1 part sample to 1 part sample diluent (1 part sample in 2 parts total volume) the dilution factor would be 2. If the diluted sample NMP22 value was found to be 80 U/mL, the reported value should be 160 U/mL (80 U/mL X 2).

C. Example Data



Calibrator Unit/ml	A490 rep1	rep2	mean	%CV
0	0.045	0.055	0.050	14.1
3	0.079	0.087	0.083	6.8
8	0.148	0.158	0.153	4.6
20	0.317	0.302	0.310	3.4
40	0.592	0.585	0.589	0.8
80	1.191	1.185	1.188	0.4
120	1.614	1.672	1.643	2.5

Samples	A490				Units/mL			
Unit/ml	rep1	rep2	mean	%CV	rep1	rep2	mean	%CV
Control 1	0.157	0.158	0.158	0.4	8.3	8.4	8.3	0.6
Control 2	0.414	0.426	0.420	2.0	27.5	28.4	27.9	2.2
Control 3	0.821	0.816	0.819	0.4	55.5	55.2	55.3	0.4
Urine Sample 1	0.459	0.453	0.456	0.9	30.7	35.8	33.3	10.8
Urine Sample 2	0.816	0.788	0.802	2.5	55.2	53.3	54.2	2.4
Urine Sample 3	1.110	1.065	1.088	2.9	74.8	71.8	73.3	2.9
Urine Sample 4	0.489	0.466	0.478	3.4	32.9	31.2	32.0	3.6

D. Acceptance of Results

1. Calibrators:
   a) The High Calibrator (120 U/mL) should have an absorbance between 0.9-2.4.
   b) The Zero Calibrator (0 U/mL) should have an absorbance less than 0.15.
2. Tri-level Controls
   a) Recovery of control concentrations should fall within the established ranges.

E. Reporting of Results

NMP22 values within the range of the standard curve may be reported to physicians. If NMP22 values are found to be greater than the highest point on the standard curve, it is recommended that the sample be diluted using the sample diluent and reassayed. The reassayed value, corrected for its dilution factor, should be reported. A referance value of 10 U/mL was found to be optimal for identifying patients at risk for occult or rapidly recurring TCC/UT in the trial reported here.

XIII. Expected Values

A prospective clinical trial was performed at 14 institutions and Matritech to determine the utility of NMP22 in identifying patients at risk for occult and rapidly recurring transitional cell carcinoma of the urinary tract (TCC/UT) 2-6 months after surgical treatment for TCC/UT. A total of 706 subjects were enrolled: 398 normal healthy volunteers, 117 subjects with benign urological diseases, 98 subjects with malignancies other than TCC/UT, and 93 subjects with TCC/UT who experienced at least 1 disease episode while enrolled in the trial. A disease episode is defined as the following: 1) performance of a surgical procedure for primary or recurring TCC/UT, such as biopsy, fulguration, or transurethral resection of a tumor, or partial cystectomy or unilateral ureteronephrectomy; 2) collection of a urine sample according to the method described in the Matritech NMP22™ Urine Collection Kit instructions, between 5 and 60 days after surgical procedure; and 3) performance of a procedure allowing assessment of presence of a neoplasm in bladder, urethra, ureters, or pelvis of the kidney, such as cystoscopic examination or total cystectomy, between 2 and 6 months after the surgical procedure. A total of 128 disease episodes occurred among 93 subjects in the trial. To determine sensitivity and specificity, the disease episodes were classified as negative (no lesions seen on cystoscopy, or if a lesion was seen, pathologic examiniation of tissue indicated no abnormality present, or atypia or dysplasia), positive (pathologic examination of tissue indicated malignancy present), or unknown (lesion seen but no tissue collected for pathologic examination).

Samples were collected from normal heathly volunteers when each subject had no symptoms of a urologic abnormality and was not under a physicians care for a urologic condition, from subjects with benign urologic conditions when the subject was receiving treatment from a physician for that condition, and from subjects with cancers other than TCC/UT when the subject was being treated or followed for that malignancy by a physician.

The relative distributions of NMP22 levels in healthy subjects,

patients with malignancies of other sites, and patients with benign diseases is presented in the following table.

Percent Distribution of NMP22 (U/mL)

	Number	NMP22 (U/mL) 0-10	>10-20	>20-50	>50-100	>100
Healthy Subject male ≥50 years	215	94.9	3.3	1.9	0	0
female ≥50 years	151	87.3	6.6	4.6	0.7	0.7
<50 years (both sexes)	32	90.6	9.4	0	0	0
TOTAL	398	91.7	5.0	2.8	0.3	0.3
Benign Diseases*						
UTI and Cystitis	26	84.6	11.5	3.8	0	0
Urinary Calculi	16	93.8	0	0	6.3	0
BPH & Prostatitis	52	92.3	7.7	0	0	0
Other	37	83.8	5.4	8.1	2.7	0
Total	117	88.0	7.7	3.4	0.9	0
Cancers other than TCC/UT						
Head and Neck	6	83.3	0	16.7	0	0
GI Tract	12	83.3	0	8.3	0	8.3
Cardiovascular & Pulmonary	12	58.3	8.3	16.7	16.7	0
Leukemia/Lymphoma	11	63.6	18.2	9.1	9.1	0
Prostate	22	81.8	0	13.6	4.5	0
Kidney (non-TCC)	18	77.8	11.1	11.1	0	0
Other	17	64.7	17.6	5.9	0	11.8
Total	98	73.5	10.2	9.2	4.1	3.1

* Some patients are included in more than one catagory.

The "other" category in the cancers other than TCC/UT includes malignancies of the bones, joints, cartilage, breast, uterine cervix, other female organs, testes, thyroid, and pheochromocytoma, and hemangiopericytoma of the leg.

In this study, 92% of the healthy subjects had concentrations of 10.0 U/mL of NMP22 or lower. Each laboratory should establish its own normal range.

Of the total 128 disease episodes (see section XIII. Expected Values for description of a disease episode) 116 could be classified as negative (no evidence of malignancy) or positive (occult or rapidly recurring malignant disease present). The first disease episode for each TCC/UT subject in the trial was also analyzed; among the 93 subjects, 87 had first disease episodes that could be classified as negative or positive. The following tables show the NMP22 results relative to a reference value of 10 U/mL for patients with occult or rapidly recurring TCC of the urinary tract, following surgical treatment for TCC/UT, and for patients with no malignant disease present.

These tables and analyses are broken down into the following categories: (1) first episode of disease (defined in Section XIII, Expected Values), (2) all episodes of disease (multiple events per patient), (3) sexes combined, (4) sexes separately, due to differences in NMP22 values seen between the sexes.

Sexes Combined

All Disease Episodes

(cystoscopic exam 2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	10	64	74
>10 U/mL	24	18	42
TOTAL	34	82	116

		95% confidence interval
sensitivity:	70.6%	55.3 - 85.9
specificity:	78.0%	69.0 - 87.0
accuracy:	75.9%	68.1 - 83.7
postive predictive value:	57.1%	42.1 - 72.1
negative predicive value:	86.5%	78.7 - 94.3

Sexes Combined

First Disease Episode

(cystoscopic exam 2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	6	46	52
>10 U/mL	19	16	35
TOTAL	25	62	87

		95% confidence interval
sensitivity:	76.0%	59.3 - 92.7
specificity:	74.2%	63.3 - 85.1
accuracy:	74.7%	65.6 - 83.8
postive predictive value:	54.3%	37.8 - 70.8
negative predicive value:	88.5%	79.8 - 97.2

Males

All Disease Episodes

(cystoscopic exam 2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	8	58	66
>10 U/mL	19	11	30
TOTAL	27	69	96

		95% confidence interval
sensitivity:	70.4%	53.2 - 87.6
specificity:	84.1%	75.5 - 92.7
accuracy:	80.2%	72.2 - 88.2
postive predictive value:	63.3%	46.1 - 80.5
negative predicive value:	87.9%	80.0 - 95.8

Males

First Disease Episode

(cystoscopic exam 2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	4	43	47
>10 U/mL	16	11	27
TOTAL	20	54	74

		95% confidence interval
sensitivity:	80.0%	62.5 - 97.5
specificity:	79.6%	68.9 - 90.3
accuracy:	79.7%	70.5 - 88.9
postive predictive value:	59.3%	40.8 - 77.8
negative predicive value:	91.5%	83.5 - 99.5

Females

All Disease Episodes

(cystoscopic exam 2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	2	6	8
>10 U/mL	5	7	12
TOTAL	7	13	20

		95% confidence interval
sensitivity:	71.4%	37.9 - 100
specificity:	46.2%	19.1 - 73.3
accuracy:	55.0%	33.2 - 76.8
postive predictive value:	41.7%	13.8 - 69.6
negative predicive value:	75.0%	45.0 - 100

Females

First Disease Episode

(cystoscopic exam 2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	2	3	5
>10 U/mL	3	5	8
TOTAL	5	8	13

		95% confidence interval
sensitivity:	60.0%	17.1 - 100
specificity:	37.5%	4.0 - 71.0
accuracy:	46.2%	19.1 - 73.3
postive predictive value:	37.5%	4.0 - 71.0
negative predicive value:	60.0%	17.1 - 100

As indicated by the above statistical analysis, urinary NMP22 values of greater than 10.0 U/mL from samples collected following a surgical procedure may indicate occult or rapidly recurring malignant disease of the urinary tract. Patients with NMP22 values below 10.0 U/mL are less likely to have malignant disease on follow-up two to six months later.
Urine NMP22 concentrations should not be interpreted as evidence of the presence or absence of malignant disease in the urinary tract without corroboration from other diagnostic procedures. Other clinically accepted tests and procedures should be considered in the diagnosis of disease and good patient management.

XIV. Performance Characteristics

A. Limit of Detection

The lowest concentration of NMP22 antigen that can be reliably measured with the Matritech NMP22(R) Test Kit is 2.1 U/mL. The minimal detectable level is defined as that NMP22 value which corresponds to the absorbance that is two standard deviations above the mean absorbance of twenty replicate determinations of the calibrator #1 (0 U/mL).

B. Precision

Following procedures outlined in the National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-T2, entitled Evaluation of Precision Performance of Clinical Chemistry Devices, within run and total precision were evaluated for three patient specimens. The three specimens were assayed in duplicate in each of two independent runs repeated daily over a 20 day period.

Specimen	Number	Mean U/mL	Within Run %CV	Total %CV
A	80	9.2	5 5	9.7
B	80	24.2	2 8	9.3
C	80	56.2	1 7	7.7

C. Recovery

Known concentrations of NMP22 from stabilized patient urine were added to stabilized urine containing low endogenous levels of NMP22. The samples were measured in duplicate. The mean of 2 assays is reported. Mean recoveries of NMP22 in stabilized urine ranged from 89% to 111% with an overall mean of 99%. An example of a typical recovery study is summarized below.

NMP22 Added (U/mL)	NMP22 Recovered (minus endogenous)	Percent Recovery
48.6	45.9	94
31.5	28.4	90
23.5	22.3	95
11.0	11.4	103

D. Linearity of Dilution

Six (6) stabilized urine samples containing elevated NMP22 levels were serially diluted with the NMP22 Urine Calibrator #1 (0 U/mL) and assayed in triplicate. Linear regression analysis of NMP22 concentrations measured versus dilution was performed. The slopes for the 6 samples ranged from 0.80 to 1.06 with a correlation coefficient of greater that 0.993, thus demonstrating that samples will dilute linearly.

E. Potentially Interfering Substances

Substances listed were evaluated and found to have no significant effects on the results of the Matritech NMP22(R) Test Kit at the following concentrations:

Urine Analytes

Substance	Concentration
Protein (Human serum albumin)	100 mg/dL
Protein (Human IgG)	100 mg/dL
Hemoglobin	1.6 mg/dL
Red Blood Cells (#/dL)	1.8×10^{11}
Whole Blood (v/v%)	1.0%
Glucose	20.0 mg/dL
Cytokeratins (TPA U/dL)	40 U/dL

Therapeutic Agents

BCG (Tice) (CFU/mL)	5.0×10^{6}
Thiotepa	60.0 mg/dL

Drugs

Digoxin	0.05 mg/dL
Acetaminophen	20 mg/dL
Sodium Ascorbate	20 mg/dL
Caffeine	20 mg/dL
Sodium Salicylate	20 mg/dL
Sodium Acetylsalicylate	20 mg/dL
Ampicillin	20 mg/dL
Tetracycline	20 mg/dL

XV. LIMITATIONS

- NMP22 concentrations should not be interpreted as evidence of the presence of malignant disease in the urinary tract without corroboration from other diagnostic procedures. Patients with known malignancy of the bladder or transitional cell carcinoma in other parts of the urinary tract may have urinary NMP22 levels within the range of concentrations observed in individuals with no known malignancy.

- Studies have shown that the release of NMP22 antigen is not constant over a 24 hour period. Therefore, a pooled sample of three separate voids collected within a 24 hour period should be tested and the results reported[10]. For details on urine collection refer to NMP22™ Urine Collection Kit Instructions for Use.

- Elevated urinary NMP22 levels have been observed in individuals with no known malignancy of the urinary tract. Occasional elevations have been observed immediately after extreme exercise (e.g. running more than ten miles) in apparently healthy individuals, in some benign urinary tract conditions (e.g. interstitial cystitis, urinary tract infections), and in patients with malignancy of any site who are undergoing systemic chemotherapy. Elevated values are always seen in patients who have undergone total cystectomy. Significance of these elevated results is unknown. Physicians should use some judgement in determining when samples are collected.

- Samples collected fewer than 5 days after an invasive procedure such as cystoscopy or catherization of the urethra may result in elevated values due to tissue damage.

- Samples collected while the patient is undergoing intravesical therapy may not accurately reflect the presence or absence of malignancy in the bladder. Interpretation of NMP22 results from these samples has not been adequately determined.

- Urinary NMP22 concentrations should only be used in conjunction with other diagnostic information in the management of patients with transitional cell carcinoma of the urinary tract.

- Only urine that has been stabilized with the NMP22 urine stabilizer should be used in this assay. Unstabilized urine and other body fluids should not be used in this assay.

XVI. Ordering Information and Technical Services

For technical assistance or to place an order contact:

Matritech Inc.
330 Nevada St.
Newton MA 02160 USA

Telephone (800) 320-2521, or (617) 928-0820
Fax (617) 928-9266

XVIII. References

1.Berezney R and D S Coffey.1974.Identification of a nuclear protein matrix. Biochem Biophys Res Commun, 60: 1410.

2.Fey EG, G Krochmalnic, and S Penman. 1986. The non-chromatin substructures of the nucleus: the ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. J Cell Biol, 102: 1654.

3.Pardoll DM, B Vogelstein, and DS Coffey. 1980. A fixed site of DNA replication in eukaryotic cells. Cell, 19: 527.

4.Zeitlin S, A Parent, S Silverstein, and A Efstratiadis. 1987. Pre-mRNA splicing and the nuclear matrix. Mol Cell Biol, 7:111.

5.Kumara-Siri MH, LE Shapiro, and MI Surks. 1986. Association of the 3,5,3e-triiodo-L-thyronine nuclear receptor with the nuclear matrix of cultured growth hormone-producing rat pituitary tumor cells (GC cells). J Biol Chem, 261: 2844.

6.Nakayasu H and R Berezney. 1989. Mapping replicational sites in the eukaryotic nucleus. J Cell Biol, 108: 1.

7.Berrios M, N Osheroff and PA Fisher. 1985. In situ localization of topoisomerase II, a major polypeptide component of the Drosophila nuclear matrix fraction. Proc Natl Acad Sci USA, 82: 4142.

8.Fey, EG and S Penman. 1988. Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci USA, 85: 121.

9.Miller TE, LA Beausang, LF Winchell and GP Lidgard. 1992. Detection of nuclear matrix proteins in serum from cancer patients. Cancer Res, 52: 422.

10.Data on file at Matritech.

For in vitro Diagnostic Use.
L0063.4 (6/96)

NMP22(R) TEST KIT PROCEDURE REFERENCE GUIDE

1. Prepare specimens and pool as outlined in pooling procedure.
2. Prepare wash solution, calibrators, controls and 2M sulfuric acid stop solution.
3. Remove required number of strip wells from foil package. Wash plate 3 times.
4. Add 200 uL/well of each calibrator, control or pooled sample into the assigned duplicated wells.
5. Incubate for 2 hours ± 5 minutes at 18-25°C. Wash plate 3 times.
6. Add 200 uL/well of DIG-AntiNMP22.
7. Incubate for 1 hour ± 5 minutes at 18-25°C. Wash plate 3 times.
8. Add 200 uL/well of HRP-SAD.
9. Incubate for 30 minutes ± 5 minutes at 18-25°C. Wash plate 3 times.
10. Prepare OPD color development solution.
11. Add 200 uL/well of OPD solution.
12. Incubate in dark area for 30 minutes ± 2 minutes at 18-25°C.
13. Add 50 uL/well of stop solution.
14. Incubate in dark area for 10 minutes at 18-25°C.
15. Read plate at 490 nm.

A. PROPRIETARY NAME

Matritech NMP22™ Urine Collection Kit
Catalog Number: D2000

Manufactured by:

MATRITECH, Inc.
330 Nevada Street.
Newton, MA 02160 USA
Phone:(617) 928-0820
(800) 320-2521
Fax: (617) 928-9266

B. KIT CONTENTS

The urine collection container contains a urine stabilizer which is a phosphate buffered saline solution with bovine derived protein stabilizers, antimicrobial agents, and bromocresol green as a colorant.

- 3 urine collection containers containing urine stabilizer
- 1 zipper lock specimen bag
- 1 absorbent pad
- 3 medicine droppers
- 1 set of patient information labels (3 small labels)
- 1 Instructions for use

C. INTENDED USE OF THIS PRODUCT

The NMP22™ Urine Collection Kit is intended for the collection, stabilization, and transport of human urine which will be tested using the Matritech NMP22® Test Kit, for *in vitro* diagnostic use, to aid in the management of patients with transitional cell carcinoma of the urinary tract (TCC/UT), after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT.

D. PRECAUTIONS

- Urine stabilizer concentrate contains fungizone™ and gentamicin as antimicrobial agents which may be toxic if ingested. Avoid contact with skin and mucous membranes. Any exposed area should be promptly and thoroughly washed.
- Store kits at a controlled room temperature of 50 to 77°F (10 to 25°C).
- Do not use kit beyond expiration date printed on the label.

E. INSTRUCTIONAL NOTES

- It is important that these insert instructions be read thoroughly and understood before using the Matritech NMP22™ Urine Collection Kit.
- Three separate urine voids must be collected within a 24-hour period.
- Each collection must be large enough in volume to reach the fill-line on the urine collection container label.
- Each large urine collection cup should be discarded after use.
- Urine samples should be collected no earlier than the 5th day following surgical treatment of the bladder or any other invasive procedure.
- All samples must be received at the clinical lab within **24 hours** of the last sample collection. If you are mailing your sample to the lab, see your urologist for proper address and overnight delivery instructions.
- If you choose to mail your sample, the box in which your collection kit was packaged should also be used for mailing the sample. Do not discard.
- If you have any questions, call your Urologist, Clinical Laboratory, or Matritech before collecting your sample.

ALL THREE SAMPLES MUST BE COLLECTED WITHIN THE SAME 24 HOUR PERIOD.

Matritech

5/96

NMP22™ Urine Collection Kit

For in vitro diagnostic use. Cat. No. D2000

Instructions for Use

NMP22™ Urine Collection Kit

Instructions for Use

Matritech

330 Nevada Street.
Newton, MA 02160
USA
Phone: (617) 928-0820
(800) 320-2521
Fax: (617) 928-9266

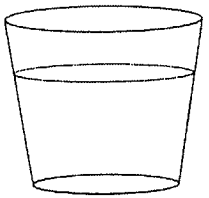
Matritech NMP22™ Urine Collection Kit

INSTRUCTIONS FOR USE

Step 1

Remove a urine collection cup from the collection kit.

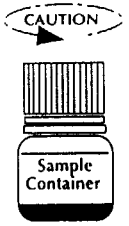
Urinate into the collection cup.



Step 2

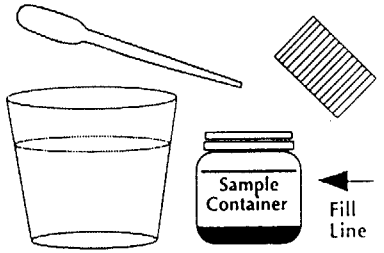
Remove one small urine collection container from the kit.

Unscrew the cap. (Be careful not to spill the blue preservative inside which may be toxic.) See precautions.



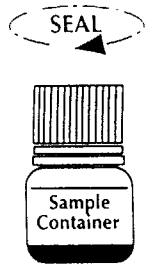
Step 3

Using one of the provided medicine droppers, transfer enough urine from the urine collection cup into the urine collection container to reach the fill-line on the label.



Step 4

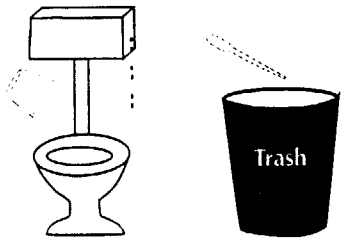
Seal the cover tightly



Step 5

Dispose of the remaining urine from the cup into the toilet or urinal.

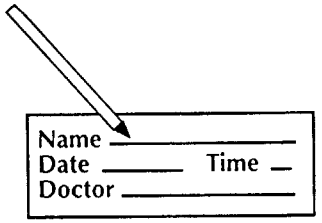
Dispose of the empty urine collection cup and medicine dropper into the trash



Step 6

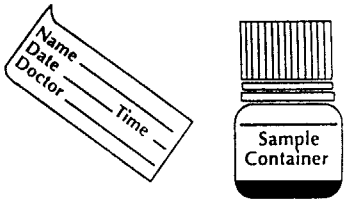
On the patient information labels provided in kit, write:

- Your Name
- Your Doctor's Name
- The Date and Time of Sample Collection



Step 7

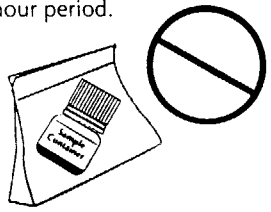
Peel the label off the adhesive backing and stick it over the existing label on the small urine collection container which contains the sample you have just collected.



Step 8

Store samples in the zipper lock bag provided and store at room temperature (50 to 77° F or 10 to 25°C) away from heat and sunlight.

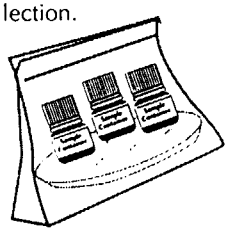
Repeat steps 1 through 7 for two more voids using a new large urine collection cup and medicine dropper within the same 24 hour period.



Step 9

Seal all three sample collections along with the absorbent pad in the zipper lock bag provided.

Deliver samples to the doctors office or clinical laboratory according to the doctor's instructions within 24 hours of last void collection.



Instructions for Use

THREE SEPARATE SAMPLES MUST BE COLLECTED WITHIN A 24 HOUR PERIOD.

If you have any questions concerning the use of this procedure, call your Urologist or Matritech, Inc. at 1-800-320-2521.

L0083 3

1. define normal range in urine of apparently healthy volunteers;

2. establish incidence of elevated NMP22 in urine of patients with active TCC/UT, examining relationship with grade of disease;

3. establish incidence of elevated urinary NMP22 in patients with malignant disease remaining in the urinary tract following surgical treatment for primary or recurrent TCC/UT, or rapidly recurring TCC/UT following surgical treatment, as evidenced by the presence of malignancy at the first follow-up monitoring procedure;

4. document effects of age, race, sex, tobacco use, and occupational carcinogen exposure on urinary NMP22 in apparently healthy subjects and subjects with different disease states.

Study site locations:

1. Boston University Hospital, Department of Urology, Boston, MA
   Boston Veterans Administration, Department of Urology, Jamaica Plain, MA
   Gennaro A. Carpinito, MD, Principal Investigator
2. University of Washington, Department of Urology, Seattle, WA
   Paul Lange, Md, Principal Investigator
3. University of Wisconsin, Division of Urology, Madison, WI
   Edward Messing, MD, Principal Investigator
4. University of Miami, Department of Urology, Miami, FL
   Mark Soloway MD, Principal Investigator
5. University of Texas Health Science Center, division of Urology, San Antonio, TX
   Michael Sarosdy, MD, Principal Investigator
6. George Washington University, Department of Hematology-Oncology, Washington, DC
   Robert Seigel, MD, Principal Investigator
7. Southern Connecticut State University, Department of Public Health, New Have, CT
   Robert Thiel, PhD, Principal Investigator
8. Cambridge Urological Associates, Cambridge, MA
   George Revervitz, MD, Principal Investigator
9. West Virginia University, Department of Urology, Morgantown, WV
   Donald Lamm, MD, Principal Investigator
10. Dupage Urology Associates, Naperville, IL
    Robert Pasciak, MD, Principal Investigator
11. University of Chicago, Joint Section of Hematology-Oncology, Chicago, IL
    Nicholas Vogelzang, MD, Principal Investigator
12. University of Chicago, Department of Urology, Chicago, IL
    Daniel Rukstalis, MD, Principal Investigator
13. Weiss Memorial Hospital, Prostate and Urology Center, Chicago, IL
    Gerald Chodak, MD, Principal Investigator

14. Harvard Surgical Associates, Jamaica Plain, MA
Paul Church, MD, Principal Investigator

Subject selection and exclusion criteria

TCC/UT Patients. All patients with transitional cell carcinoma of the urinary tract or bladder cancer of other histology were eligible to participate in this study provided there was documented evidence of active disease within the eighteen months prior to entry into the study and the patient had no prior or concomitant malignancy of a different site, except basal or squamous cell carcinoma of the skin. This last exclusion criterion did not include patients with prostate cancer who were diagnosed incidental to removal of the prostate as part of a total cystectomy and who had no clinical signs or symptoms of prostate malignancy prior to the total cystectomy. Patients with all stages and grades of disease were eligible for inclusion in the trial.

Patients with benign conditions of the urinary tract. Any patient with a benign condition of the genitourinary tract with definitive diagnosis in which malignancy had been ruled out, and who had no prior or concomitant diagnosis of malignancy, other than basal cell or squamous cell carcinoma of the skin, was eligible for inclusion in the trial.

Patients with malignancies other than TCC/UT. Any patient with a malignancy other than TCC/UT with active disease within the 18 months prior to entry into the study, who had no prior or concomitant diagnosis of malignancy, except basal or squamous cell carcinoma of the skin other than the malignancy qualifying them for entry into the study, was eligible for inclusion in the trial.

Normal Healthy Volunteers. Subjects with no known conditions of the urinary tract or malignancy, other than basal or squamous cell carcinoma of the skin, age 50 or over, were eligible for inclusion in the trial. An attempt was made to recruit volunteers in a manner to approximate the distribution of TCC/UT patients with regard to age, sex, race, and smoking habits. These subjects were self-screened, that is, no requirement was made that volunteers be medically examined to rule out urological disease. A small group of normal healthy volunteers under age 50 were included to test for NMP22™ differences due to age.

Study Population

Three hundred forty-two normal healthy volunteers age fifty or older, 33 normal healthy volunteers age under age fifty, 117 subjects with benign urological conditions, 98 patients with malignancies other than TCC/UT, and 204 subjects with TCC/UT were included in the analysis

Results and Analysis of Study

Normal Healthy Volunteers. Table 3 shows the distribution of NMP22™ values for normal healthy volunteers. Demographic conditions that were examined include: age, sex, and race. The only demographic characteristics for which differences in NMP22 levels were found are sex, age, and carcinogen exposure. There was a statistically significant difference between males and females (Md = 2.46 U/mL for males and 3.92 U/mL for females) and between subjects less than 50 years vs subjects 50 or older (Md = 5.66 U/mL for subjects less than 50 and 2.78 U/mL for subjects 50 or older). In order to determine which age-sex groups were affected, a Friedman Chi-square, controlled for sex, was performed, indicating no difference in the age group medians. There was a significant difference in NMP22™ values between normal healthy males and females. This difference explained by the variation seen among females within various age groups.

TABLE 3 NORMAL HEALTHY VOLUNTEERS

Descriptive Statistics for NMP22 values by age, sex, and race

Subject group	n	mean ± SE	median	95% confidence interval on median	%<10 U/
all subjects	398	5.00 ± 0.44	2.90	2.35 - 3.45	91.8
age <50	32	5.61 ± 0.59	5.66	2.92 - 7.27	90.6
50 ≤60	85	4.01 ± 0.53	2.88	2.31 - 3.51	96.5
60 ≤70	174	3.78 ± 0.35	2.33	2.14 - 2.83	94.8
≥70	107	7.58 ± 1.44	3.40	2.84 - 4.23	83.2
all males	225	3.74 ± 0.35	2.46	2.20 - 2.73	94.7
age <50	10	4.53 ± 1.06	2.86	2.46 - 5.76	90.0
50 ≤60	38	3.71 ± 0.80	2.60	2.12 - 3.47	97.4
60 ≤70	103	2.86 ± 0.29	2.12	1.80 - 2.28	97.1
≥70	74	4.88 ± 0.87	2.92	2.24 - 3.40	90.5
all females	173	6.64 ± 0.89	3.92	3.09 - 4.53	87.9
age <50	22	6.10 ± 0.70	6.14	3.46 - 7.55	90.9
50 ≤60	47	4.26 ± 0.70	2.93	2.32 - 4.15	95.7
60 ≤70	71	5.12 ± 0.73	3.08	2.39 - 4.07	91.5
≥70	33	13.63 ± 4.09	5.99	3.88 - 8.35	66.7
African-American	37	9.89 ± 3.66	3.19	2.23 - 4.78	86.5
White	343	4.57 ± 0.32	2.88	2.48 - 3.28	91.8
Hispanic	16	3.07 ± 0.55	2.40	1.11 - 3.15	100.0

The effect of presence of the following medical conditions in the normal healthy volunteers was studied: diabetes, autoimmune conditions, cardiovascular conditions, kidney dysfunction, and other non-urological conditions. None of these conditions had a statistically significant effect on NMP22™ levels.

Subjects with benign urinary tract conditions. The following benign urinary tract conditions were benign prostatic hyperplasia (BPH), prostatitis, urinary calculi, urinary tract infection (UTI) and interstitial cystitis, and benign conditions not included in categories above. Table 4 shows the distribution of NMP22 values for these groups. Some subjects had more than one benign condition and consequently were included in more than one group

Kruskal-Wallis non-parametric analysis of variance was performed comparing NMP22™ levels from subjects with various benign conditions to normal healthy volunteers of the appropriate sex group. The analysis indicated that, in this group of subjects, benign urinary tract conditions did not cause significantly elevated NMP22 levels.

Subjects with cancers other than TCC/UT. Table 4 also summarizes the NMP22 values for this group. When taken altogether this group had NMP22 values that were significantly different from normal healthy subjects, as determined by the Mann-Whitney U test. There were 73 males in this group with a median NMP22 level of 4.12 U/mL, and 25 females, with a median NMP22 level of 6.33 U/mL, as compared to the normal healthy group with medians of 2.38 U/mL for males and 3.90 U/mL for females. For every cancer site that was examined separately, except

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prostate and GI tract, either males or females or both had NMP22 values that were significantly higher than the normal healthy volunteers group.

Percent distributions of NMP22 levels in healthy subjects, patients with malignancies of other sites, and patients with benign diseases is shown in Table 4. A negative NMP22™ result is ≤ 10 U/mL.

TABLE 4

Percent Distribution of NMP22 (U/mL)

	Number	0-10	>10-20	>20-50	>50-100	>100
Healthy Subjects						
male ≥50 years	215	94.9	3.3	1.9	0.0	0.0
female ≥50 years	151	87.3	6.6	4.6	0.7	0.7
< 50 years (both sexes)	32	90.6	9.4	0.0	0.0	0.0
TOTAL	398	91.7	5.0	2.8	0.3	0.3
Benign Diseases*						
UTI and Cystitis	26	84.6	11.5	3.8	0.0	0.0
Urinary Calculi	16	93.8	0.0	0.0	6.3	0.0
BPH & Prostatitis	52	92.3	7.7	0.0	0.0	0.0
Other	37	83.8	5.4	8.1	2.7	0.0
TOTAL	117	88.0	7.7	3.4	0.9	0.0
Cancers other than TCC/UT						
Head and Neck	6	83.3	0.0	16.7	0.0	0.0
GI Tract	12	83.3	0.0	8.3	0.0	8.3
Cardiovascular & Pulmonary ..	12	58.3	8.3	16.7	16.7	0.0
Leukemia/ Lymphoma ...	11	63.6	18.2	9.1	9.1	0.0
Prostate	22	81.8	0.0	13.6	4.5	0.0
Kidney (non-TCC)	18	77.8	11.1	11.1	0.0	0.0
Other	17	64.7	17.6	5.9	0.0	11.8
TOTAL	98	73.5	10.2	9.2	4.1	3.1

*Some patients are included in more than one category.

The "other" catagory in the cancers other than TCC/UT included malignancies of the bones, joints, cartilage, breast, uterine cervix, other female organs, testes, thyroid, and pheochromocytoma, and hemangiopericytoma of the leg.

Subjects with TCC/UT. Two hundred four subjects with TCC/UT, who fulfilled all of the study criteria, were entered into the trial with adequate documentation and submitted at least one urine sample. Of these, 94 subjects experienced a disease episode, which was defined as an episode of TCC/UT or bladder cancer of other histology within the 18 months prior to enrollment; including all stages and grades of disease. The patients were then evaluated by:

1) Performance of a surgical procedure for primary or recurrent TCC/UT, including biopsy, fulguration, or transurethral resection of a tumor of the bladder, urethra, ureters, or pelvis of the kidney, or partial cystectomy or unilateral ureteronephrectomy;

2) Collection of a urine sample, according to the method described in the Protocol for this trial, no less than 5 days after the surgical procedure; and

3) Performance of a procedure, 30 to 180 days after the surgical procedure, allowing assessment of presence of a neoplasm in the bladder, urethra, ureters, or pelvis of the kidney, such as cystoscopic examination or total cystectomy, after collection of the urine sample.

Of the total 128 disease episodes 116 could be classified as negative (no evidence of malignancy) or positive (occult or rapidly recurring malignant disease present). The first disease episode for each TCC/UT subject in the trial was also analyzed; among the 93 subjects, 87 had first disease episodes that could be classified as negative or positive. Table 5 shows the NMP22 results relative to a reference value of 10 U/mL for patients with occult or rapidly recurring TCC of the urinary tract, following surgical treatment for TCC/UT, and for patients with no malignant disease present.

The data in Table 5 were analyzed in the following categories:

1. First episode of recurrence; i.e., performance of a surgical procedure for primary or recurrent TCC/UT, including biopsy, fulguration, or transurethral resection of a tumor.

2. All disease episodes of recurrence, multiple events per patient.

3. Sexes Combined.

4. Sexes separately, due to the differences of NMP22™ seen between the sexes.

TABLE 5

Sexes combined

All Disease Episodes

(2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	10	64	74
>10 U/mL	24	18	42
TOTAL	34	82	116

sensitivity:	70.6%
specificity:	78.0%
accuracy:	75.9%
pos predictive value:	57.1%
neg predictive value:	86.5%

First Disease Episode

(2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	6	46	52
>10 U/mL	19	16	35
TOTAL	25	62	87

sensitivity:	76.0%
specificity:	74.2%
accuracy:	74.7%
pos predictive value:	54.3%
neg predictive value:	88.5%

Males

All Disease Episode

(2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	8	58	66
>10 U/mL	19	11	30
TOTAL	27	69	96

sensitivity:	70.4%
specificity:	84.1%
accuracy:	80.2%
pos predictive value:	63.3%
neg predictive value:	87.9%

First Disease Episode

(2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	4	43	47
>10 U/mL	16	11	27
TOTAL	20	54	74

sensitivity:	80.0%
specificity:	79.6%
accuracy:	79.7%
pos predictive value:	59.3%
neg predictive value:	91.5%

Females

All Disease Episode

(2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	2	6	8
>10 U/mL	5	7	12
TOTAL	7	13	20

sensitivity:	71.4%
specificity:	46.2%
accuracy:	55.0%
pos predictive value:	41.7%
neg predictive value:	75.0%

First Disease Episode

(2 to 6 Months after Surgical Treatment)

NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	2	3	5
>10 U/mL	3	5	8
TOTAL	5	8	13

sensitivity:	60.0%
specificity:	37.5%
accuracy:	46.2%
pos predictive value:	37.5%
neg predictive value:	60.0%

As indicated from the above analysis, urinary NMP22 values of greater than 10.0 U/mL from samples collected following a surgical procedure may indicate occult or rapidly recurring malignant disease of the urinary tract. Patients with NMP22 values below 10.0 U/mL were less likely to have malignant disease on follow-up two to six months later. Urine NMP22 concentrations should not be interpreted as evidence of the presence or absence of malignant disease in the urinary tract without corroboration from other diagnostic procedures. Other clinically accepted tests and procedures should be considered in the diagnosis of disease and good patient management.

VIII. Conclusions Drawn from the Studies

Subjects were enrolled in this study in a manner to ensure reasonable distributions by clinical status in the TCC/UT patient group and by demographic variables in the normal healthy volunteer group. Adequate numbers were enrolled in each group to detect significant differences in NMP22™ levels among groups, if such differences existed. The study was conducted in a blinded manner; that is, the investigators at the clinical sites did not know the NMP22 results for the subjects they had enrolled, and the personnel assaying the samples did not know the subject group or clinical status of the subjects who had contributed the samples.

Safety and effectiveness of this device was based on gender, race and ethnicity. Differences in the sensitivity and specificity of some groups were found. The results of the clinical investigations are as reported in the package labeling.

Since these data were not normally distributed, all statistical analyses were performed using nonparametric statistical techniques. The Mann-Whitney U test was used for comparisons between two groups, and the Kruskal-Wallis one-way analysis of variance was used for comparisons among more than two groups.

There were no publications or other known investigations of the clinical utility of this analyte in urine for comparison with the results reported here.

Performance specifications for the Matritech NMP22™ Test Kit met the usual requirements for an immunoassay to be performed in a licensed clinical laboratory, including reproducibility, precision, recovery, stability, linearity, and analytical sensitivity.

The findings indicated that NMP22™ had potential use as an aid to the urologist planning care of patients with TCC/UT, in particular, following surgical treatment to evaluate that patient's needs for closer monitoring and perhaps more aggressive treatment. An NMP22 value less than 10 U/mL indicated that a patient had lower risk for presence of malignancy 3 to 6 months after treatment.

An elevated NMP22 value (>10 U/mL) following surgery did not necessarily indicate that patient would have active malignant disease at follow-up 3 to 6 months later (78.5 percent specificity in the patient group). Further follow-up of these patients would be necessary to determine if elevated values in some patients indicated that clinically evident disease would be detected on subsequent examination. Other conditions may cause elevations in this parameter, particularly some benign urinary tract conditions, such as urinary tract infections

cell carcinoma of the urinary tract (TCC/UT) after surgical treatment to identify those patients at risk for occult or rapidly recurring TCC/UT.

Risk/Benefit Analysis

Since the Matritech NMP22™ Test Kit is not intended for use as a diagnostic tool without other clinical and diagnostic data, patients will not be treated solely on the basis of results of this test. The physician will use this test to help determine the need for more or less aggressive monitoring of patients with TCC/UT and will base treatment decisions on the outcome of currently accepted standard of practice such as cystoscopic examination or imaging procedures. Therefore the risk to the patient of inappropriate or inadequate treatment based on the NMP22 assay is low, but the benefit of identifying patients with higher risk of presence of malignancy 3 to 6 months after surgical treatment is increased.

IX. Panel recommendation

The Immunology Device Panel recommended at the panel meeting on November 30, 1995 that the PMA for the Matritech NMP22™ Test Kit be approved with conditions and recommended the following:

1. A further analysis of the difference between the sexes in the verify the cut off of 10 u/mL.

2. Provide additional data on African American males in the list groups.

X. CDRH Action on the Application

CDRH concurred with the recommendation of the Panel. CDRH issued an approvable letter on December 20, 1995 requesting the additional information to satisfy the conditions listed by the Panel. Matritech, Inc. Responded in the form of an Amendment on February 6, 1996 and are included in the above data analysis. Therefore it is reasonable to conclude that the benefits of use of the NMP22™ Test Kit for the targeted population outweigh the risk of illness or injury when used as indicated in accordance with the directions for use.

CDRH issued an approval order for the applicant's PMA for the Matritech NMP22™ Test Kit on July 2, 1996.

The applicant's manufacturing and control facilities were inspected on March 25,1996 and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMP). The shelf-life of the Matritech NMP22™ Test Kit has been established at 15 months.

XI. Approval Specifications

Directions for use: See attached labeling

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order (Attachment B).

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MATRITECH INC.

MATRITECH NMP22(R) TEST KIT

Store at 2-8°C

Instructions for Use

For the in vitro quantitative measurement of the nuclear matrix protein NMP22 in stabilized urine as as aid in the management of patients with transitional cell carcinoma of the urinary tract(TCC/UT), after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT

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I. Product Information

Matritech NMP22(R) Test Kit
Manufactured by Matritech Inc., Newton MA 02160, USA
Catalog Number D1100
96 Determinations

Caution: Federal law restricts this device to sale and distribution by or on the order of a physician or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

II. Intended Use

The Matritech NMP22(R) Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of the nuclear matrix protein NMP22 in stabilized voided urine. The Matritech NMP22(R) Test Kit is indicated as an aid in the management of patients with transitional cell carcinoma of the urinary tract (TCC/UT), after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT.

III. Contraindications, Warnings, and Precautions

A. Contraindications

1. There are no known contraindications for the Matritech NMP22(R) Test Kit.

B. Precautions

1. For in vitro diagnostic use only.
2. **Specimens used in this kit MUST be collected using the Matritech NMP22™ Urine Collection Kit and stabilized with the NMP22 Stabilizer according to Collection Kit directions for use.**
3. Do not mix components from different kit lots.
4. Do not use components after the expiration date.
5. Use only **plastic** containers to process or store urine samples, calibrators or controls. Do not use any glass containers other than containers in which calibrators and controls are supplied.
6. For disposal of any remaining kit reagents or specimens refer to local regulations for the proper disposal of medical waste.
7. Store the Matritech NMP22™ Urine Collection Kit at a controlled room temperature of 50 to 70°F (10 to 25°C).
8. Do not use the Matritech NMP22™ Urine Collection Kit beyond the expiration date printed on the label.

C. Warnings

1. HANDLE ALL SPECIMENS AS IF CAPABLE OF TRANSMITTING INFECTION.
2. Do not eat, drink, or smoke where kit reagents are being handled.
3. Do not pipette by mouth.
4. Wear disposable gloves, laboratory coats and other appropriate protective devices while handling samples and kit reagents. Thoroughly wash hands afterwards.
5. The calibrators and controls in this kit contain human albumin. Each lot of albumin is tested and found to be negative for antibody to human immunodeficiency virus (HIV) and Hepatitis B Surface Antigen (HBsAg). Always handle all human derived materials as if they are potentially infectious.
6. OPD tablets and stopping reagent should be handled carefully because they are skin and mucous membrane irritants. If contact with skin or mucous membrane occurs, wash immediately with water.
7. The stabilizer found in the Urine Collection Container of the Matritech NMP22™ Urine Collection Kit contains fungizone™

and gentamicin as antimicrobial agents which may be toxic if ingested. Any exposed area should be promptly and thoroughly washed.

IV. Summary and Explanation of the Test

Nuclear matrix proteins (NMPs) make up the internal structural framework of the nucleus[1,2] and are associated with such functions as DNA replication, RNA synthesis, and hormone binding[3,4,5]. Further work has indicated that NMPs are involved in regulation and coordination of gene expression[4,6,7]. Later work by Fey and Penman[8] demonstrated that NMP expression varied with cell type of origin. This observation was followed by work showing that soluble NMPs could be detected in the serum of cancer patients in higher concentrations than were found in normal sera[9]. Most recently, Partin and colleagues demonstrated that the pattern of expression of NMP differed in normal prostate tissue, benign prostatic hyperplasia, and prostate cancer. Previous work has identified specific NMPs for osteosarcoma, colon and breast cancer.

The antibodies contained within this assay recognize the head and rod domains of NuMA, nuclear mitotic apparatus protein. NuMA has been shown to be present in malignant tissues at levels more than ten times higher than in normal tissues[10]. The NuMA antigen moiety detected by the Matritech NMP22(R) Test Kit is referred to as NMP22. The assay detects both complexed (> 100 kD) and fragmented (~30 kD) forms of NuMA in stabilized voided urine.
In the urine of healthy individuals, NMP22 is present at low levels. Patients with TCC/UT present in the urinary tract have been shown to release higher levels of NMP22 into the urine. The assay is designed to quantify NMP22 in stabilized voided urine.

V. Principle of Matritech NMP22(R) Test Kit

The Matritech NMP22(R) Test Kit is an easy-to-use enzyme immunoassay in a 96 well microplate strip-well format. The assay employs two monoclonal antibodies that are specific for the nuclear matrix protein NMP22. Calibrators, controls and stabilized patient urine samples are reacted with an antibody coated onto wells of a microplate. After washing, the captured NMP22 antigen is reacted with a second antibody labeled with digoxigenin (DIG). After a wash, the digoxigenin-labeled antibody is detected with an anti-digoxigenin antibody coupled to horseradish peroxidase (HRP-SAD) using O-phenylenediamine (OPD) substrate. The reaction is terminated by the addition of 2 molar sulfuric acid (2M H_2SO_4). The concentration of antigen in the urine is proportional to the intensity of color development and the actual concentration is determined from a standard curve. The standard curve is determined by the concurrent testing of the

NMP22 Calibrators which range in concentration from 0 to approximately 120 U/mL. The actual calibrator values are printed on the calibrator vial labels.

VI. Reagents

A. Materials Supplied

1. **7 NMP22 Urine Calibrators**; Lyophilized
(2 mL after reconstitution)
After reconstitution with 2 mL of deionized water, each vial contains NMP22 Antigen (except calibrator #1) in a synthetic human urine solution containing human albumin, with bovine proteins and preservatives. Calibrators range in concentration from 0 to approximately 120 U/mL. The actual calibrator value is printed on the calibrator vial label. Store at 2-8°C.

2. **3 NMP22 Urine Controls**; Lyophilized
(2 mL after reconstitution)
After reconstitution with 2 mL of deionized water each vial contains NMP22 antigen in a synthetic human urine solution containing human albumin with bovine proteins, and preservatives. See each vial for assigned ranges. Store at 2-8°C.

3. **1 NMP22 Coated Microplate Strip Well Plate**
Each plate consists of twelve 8-well strips coated with mouse monoclonal anti-NMP22. Store strips at 2-8°C in the sealed foil pack provided, with desiccant.

4. **1 Digoxigenin Anti-NMP22 Reagent**; 20 mL
Each vial contains digoxigenin-labeled mouse monoclonal antibody with goat, mouse, and bovine serum proteins and preservative. Store at 2-8°C.

5. **1 HRP-SAD Reagent**; 20 mL
Each vial contains horseradish peroxidase labeled sheep anti-digoxigenin antibody (HRP-SAD) with goat, mouse, and bovine serum proteins and preservative. Store at 2-8°C.

6. **1 Sample Diluent**; Lyophilized
(10 mL after reconstitution)
After reconstitution with 10 mL of deionized water each vial contains a synthetic human urine solution containing human albumin with bovine proteins and preservatives. Store at 2-8°C.

7. **1 Color Development Buffer**; 40 mL
Each vial contains a solution of hydrogen peroxide in a citrate-phosphate buffer with preservatives. Store at 2-8°C.

8. **OPD Tablets**; 4 tablets
Individually foil-wrapped tablets containing O-Phenylenediamine and excipients. Store at 2-8°C.

9. **1 Wash Solution 100X Concentrate**; 30 mL
Each vial contains a 100 X concentrate of a non-ionic detergent in phosphate buffered saline. Store at 2-8°C.

10. **Instruction for Use.**

B. Reagents Required but not Provided

Matritech NMP22™ Urine Collection Kit, Catalog Number D2000

2M Sulfuric Acid Stop Solution; see section XI.A.5. for preparation instructions.

C. Other Materials Not Provided

- 200 uL precision pipette with disposable tips
- 1000 uL precision pipette with disposable tips
- Microplate plate reader to measure absorbance at 490 nm
- Microplate plate washing and aspiration device; (capable of delivering at least three wash/aspiration cycles with a dwell time of 10 seconds)
- Refrigerated centrifuge
- Plastic test tubes to prepare OPD solution
- Plastic test tubes for centrifuging, pooling, or diluting urine specimens prior to use
- Blank strip wells

VII. Storage Instructions

- Store Kit Components at 2 to 8°C immediately upon receipt.
- Components as packaged are stable through the expiration date printed on the kit box label.
- Keep foil packaging for OPD tablets sealed until ready for use.
- All reagents must be brought to room temperature (18-25°C) prior to use. Immediately after use, all reagents except the diluted Wash Solution should be stored at 2-8°C.
- NMP22 Urine Calibrators, Controls and Sample Diluent, when reconstituted with 2 mL (calibrators and controls) or 10 mL (sample diluent) of deionized water are stable for 14 days when stored at 2-8°C.